MafB boosts osteoclastogenesis: A break-through to the current treatment for Multicentric Carpal Tarsal Osteolysis(多中心性手根骨足根骨融解症（MCTO）の治療を目的とした、破骨細胞における転写因子MafBの機能解析)

内容記述	この博士論文は内容の要約のみの公開（または一部非公開）になっています
year	2019
学位授与大学	筑波大学 (University of Tsukuba)
学位授与年度	2018
報告番号	12102甲第9211号
URL	http://hdl.handle.net/2241/00157891

School of Integrative and Global Majors
Ph.D. Program in Human Biology (HBP)

論文概要

Dissertation Abstract

Title of Doctor Dissertation:

MafB boosts osteoclastogenesis: A break-through to the current treatment for Multicentric Carpal Tarsal Osteolysis

(多中心性手根骨足根骨融解症(MCTO)の治療を目的とした、破骨細胞における転写因子MafBの機能解析)

Last or Family Name	First	Middle
Tsunakawa	Yuki	

Student Number s1430588@u.tsukuba.ac.jp

Primary Academic Advisors

Affiliation: Faculty of Medicine, Division of Anatomy and Embryology

Name: Satoru Takahashi

Abstract

Purpose:

Multicentric carpal tarsal osteolysis (MCTO) is a rare skeletal disease with progressive osteolysis at the carpal and tarsal bones. Zankl A *et al.,* defined an autosomal missense mutation in the transactivation domain of transcription factor *MAFB* as the cause of MCTO, which is also reported to be highly expressed in osteoclasts. However, there is no definite treatment for MCTO today since the relationship between MafB and osteoclasts *in vivo* remains unknown. Previous studies show that MafB is highly expressed in myeloid cells including osteoclasts and that MafB negatively regulates osteoclastogenesis *in vitro*. Therefore, it was predicted that MCTO occurs from the loss of MAFB function, which causes upregulation of osteoclast differentiation. However, the function of MafB in osteoclastogenesis *in vivo* is not clarified till this day. Thus, function of MafB in osteoclastogenesis *in vivo* needs more clarification using animal models for a better understanding and develop a treatment against MCTO. The aim of this study is to clarify the function of MafB in osteoclast differentiation and cause a break-through in the treatment of MCTO.

Materials and Methods:

Myeloid specific *Mafb* knock-out mice (*Mafb*$^{f/f}$*::*LysM-Cre) were used in this study to examine the function of MafB in osteoclasts. The effect of osteoclatogenesis against the bone phenotypes of these mice were examined by measuring the bone density of *Mafb*$^{f/f}$*::*LysM-Cre mice at 8 weeks using micro-CT analysis. In addition, resistance against age-related osteoporosis and RANKL-induced bone loss was also analyzed using micro-CT analysis. Osteoclatogenesis ability of the bone marrow cells of *Mafb*$^{f/f}$*::*LysM-Cre mice was measured

by primary osteoclast differentiation assay and pit assay. Moreover, RNA-sequencing was conducted to analyze the candidate pathways that MafB could be regulating during osteoclast differentiation. Following these findings, mouse harboring the human MCTO mutation (*Mafb*$^{MCTO/MCTO}$) was generated using the CRISPR-Cas9 system to relate our findings from *Mafb*$^{f/f}$*::*LysM-Cre mice to the pathology. Bone phenotype of femurs and tarsal bones was analyzed through micro-CT. To analyze the effect of MCTO on the MafB protein itself, transactivation level of MCTO mutated MafB was measured using luciferase assay against known MafB target promoters. Finally, MCTO mutation was related to osteoclastogenesis ability of *Mafb*$^{MCTO/MCTO}$ mice, by primary osteoclast differentiation assay using bone marrow cells of *Mafb*$^{MCTO/MCTO}$ mice.

Results:

Micro-CT analysis of the femur bone of *Mafb*$^{f/f}$*::*LysM-Cre mice showed an increase in bone density compared to control, indicating that MafB promotes osteoclast differentiation *in vivo*. This was further confirmed by *Mafb*$^{f/f}$*::*LysM-Cre mice exhibiting resistance against osteoclast driven bone resorption. Culturing osteoclasts from bone marrow cells of *Mafb*$^{f/f}$*::*LysM-Cre mice showed lower numbers of multinucleated cells compared to control, especially at 42 hours after RANKL-induction. To identify the pathway that MafB regulates in osteoclastogesis, RNA-sequencing was performed against *Mafb*$^{f/f}$*::*LysM-Cre mice derived osteoclasts at 42 hours after RANKL-induction. Osteoclast inhibitory genes and markers of other immune cells including macrophages, were up-regulated in *Mafb*$^{f/f}$*::*LysM-Cre mice derived osteoclasts. From this, it was predicted that MafB promotes osteolastogenesis by fine tuning osteoclast precursors to the osteoclast linage. However, the results obtained from *Mafb*$^{f/f}$*::*LysM-Cre mice contradicts to the symptoms of MCTO patients. Therefore, the bone phenotypes of *Mafb*$^{MCTO/MCTO}$ mice were analysed though micro-CT. The bone density of femur bones and tarsal bone volume was comparable between control and *Mafb*$^{MCTO/MCTO}$ mice at 2 weeks. However, both femur bone density and tarsal bone volume were significantly lower in *Mafb*$^{MCTO/MCTO}$ mice by 8 weeks. The function of MCTO mutated MafB were also examined by luciferase assay against reported target genes of MafB, where MCTO mutated MafB increased transcriptional ability compared to MafB containing the intact protein sequence. From the *in vivo* phenotype of *Mafb*$^{MCTO/MCTO}$ mice, it was suggested *Mafb*$^{MCTO/MCTO}$ mice increases osteoclast differentiation. Therefore, osteoclast differentiation was assessed using primary osteoclast differentiation assay using *Mafb*$^{MCTO/MCTO}$ mice derived cells. As a result, *Mafb*$^{MCTO/MCTO}$ mice showed increased multinucleated cell number compared to control, suggesting that MCTO occurs from hyper-differentiation of osteoclasts.

Discussion:

Tis study, demonstrates the function of MafB in osteoclastogenesis *in vivo* for the first time. These include; MafB promotes the differentiation of osteoclasts and MCTO mutation activates transcriptional function of MafB, and MCTO is caused by over-differentiating osteoclasts. These were shown by *Mafb*$^{f/f}$*::*LysM-Cre mice being resistance against osteoclast driven bone resorption and primary osteoclast differentiation experiments showing lower multinucleated osteoclasts. RNA-sequencing results also revealed an “identity crisis” of osteoclast progenitors from *Mafb*$^{f/f}$*::*LysM-Cre mice at 42 hours post culture. These results suggest that MafB regulates

osteoclastogenesis at later stages of differentiation by priming osteoclast progenitors to the osteoclast linage. Although previous studies demonstrate that MafB works as a negative regulator against osteoclastogenesis, they also mention that MafB expression gradually increases its expression after RANKL induction. In contrast, this study may be focusing on the MafB function at later stages of osteoclastogenesis, which acts as a positive regulator rather than an inhibitor. Therefore, the combination of the study of MafB as an inhibitor of osteoclatogenesis and the current study can provide valuable information to the whole picture of MafB in osteoclast differentiation.

In order to relate the findings from the *Mafb*$^{f/f}$*::*LysM-Cre mice to MCTO, bone phenotypes of *Mafb*$^{MCTO/MCTO}$ mice were analyzed in a time course manner. The bone density of femur bones and tarsal bone volume was comparable between control and *Mafb*$^{MCTO/MCTO}$ mice at 2 weeks. However, by 8 weeks there were less femur bone density and tarsal bone volume in *Mafb*$^{MCTO/MCTO}$ mice. These results suggest that MCTO onsets between 2 weeks and 8 week-of-age.

Since the *in vivo* bone phenotypes of *Mafb*$^{MCTO/MCTO}$ mice presented osteolysis-like phenotype, the transactivation function of MCTO-mutated MafB was examined. Luciferase assay results against MafB target promoters showed that transactivation function increases in MCTO-mutated MafB. These results indicate that MCTO mutation on MafB also promotes osteoclastogenesis, since *Mafb*-depletion inhibited osteoclastogenesis in *Mafb*$^{f/f}$*::*LysM-Cre mice. Primary osteoclast differentiation assay using *Mafb*$^{MCTO/MCTO}$ mice derived cells supported this hypothesis, where multinucleated osteoclast number were increased in cultures of *Mafb*$^{MCTO/MCTO}$ mice derived cells. These results suggest that MCTO happens from increased MafB function, which in turn causes osteoclast hyper-activity. Although these results show that MCTO could happen from osteoclast differentiation, the direct contribution of osteoclasts to MCTO *in vivo* remains to be answered. Therefore, more investigation through histological examination and rescue experiment using bisphosphonate at the timing of onset must be conducted to truly validate our hypothesis.

Over all, the present study states that MafB holds another function other than inhibiting osteoclastogenesis, and that MCTO occurs from promoted osteoclast differentiation. These findings may facilitte the progression of drug development that targets osteoclasts in MCTO patients